

# AMPA receptors mediate the pro-cognitive effects of electrical and optogenetic stimulation of the medial prefrontal cortex in antidepressant non-responsive Wistar–Kyoto rats

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## Abstract

**Background:** The chronic mild stress (CMS) procedure is a widely used animal model of depression, and its application in Wistar–Kyoto (WKY) rats has been validated as a model of antidepressant-refractory depression. While not responding to chronic treatment with antidepressant drugs, WKY rats do respond to acute deep brain stimulation (DBS) of the medial prefrontal cortex (mPFC). In antidepressant-responsive strains there is evidence suggesting a role for AMPA subtype of glutamate receptor in the action mechanism of both antidepressants and DBS.

**Methods:** Animals were subjected to CMS for 6 to 8 weeks; sucrose intake was monitored weekly and novel object recognition (NOR) test was conducted following recovery from CMS. Wistars were treated chronically with venlafaxine (VEN), while WKY were treated acutely with either DBS, optogenetic stimulation (OGS) of virally-transduced (AAV5-hSyn-ChR2-EYFP) mPFC or ventral hippocampus, or acute intra-mPFC injection of the AMPA receptor positive allosteric modulator CX-516. The AMPA receptor antagonist NBQX was administered, at identical sites in mPFC, immediately following the exposure trial in the NOR.

**Results:** Sucrose intake and NOR were suppressed by CMS, and restored by VEN in Wistars and by DBS, OGS, or CX-516 in WKY. However, OGS of the ventral hippocampal afferents to mPFC was ineffective. A low dose of NBQX selectively blocked the procognitive effect of VEN, DBS and OGS.

**Conclusions:** These results suggest that activation of AMPA receptors in the mPFC represents a common pathway for the antidepressant effects of both conventional (VEN) and novel (DBS, OGS) antidepressant modalities, in both antidepressant responsive (Wistar) and antidepressant-resistant (WKY) rats.

## Keywords

Chronic mild stress, AMPA receptors, medial prefrontal cortex, deep brain stimulation, optogenetic stimulation, venlafaxine, ampakine, Wistar–Kyoto rat

## Introduction

Depression is a major cause of ill health and economic burden, reflecting the high proportion of individuals with comorbid psychiatric disorders or other severe health challenges, and the persistence and severity of their symptoms (Ferrari et al., 2013; Lépine and Briley, 2011). Indeed, while neuropsychiatric disorders in general carry by far the greatest global burden of disease, the largest single burden is carried by people with major depression (Ferrari et al., 2013; Greden, 2001), which is projected to become the leading cause of global disease burden by 2030 (Lépine and Briley, 2011). The most severely disabled group are those with treatment-resistant depression (TRD) (Greden, 2001), who comprise more than half of all depressed patients (Nemeroff, 2007; Thase, 2011; Thomas et al., 2013).

There have been significant recent advances in understanding of the pathophysiology of depression, much of it based on the use of animal models of depression (Belzung et al., 2015; Willner et al., 2013). However, the fact that responsiveness to antidepressant drug treatment has been considered to be an essential feature of a valid animal model of depression (Willner, 1984), severely limits their usefulness in relation to TRD (Hendrie et al., 2013;

Willner and Belzung, 2015), which requires alternative models in which conventional antidepressants are ineffective (Willner and Belzung, 2015; Willner et al., 2013, 2014). The opportunity to develop such models was afforded by the clinical discovery of novel treatments that bring about rapid and sometimes lasting improvements in a high proportion of TRD patients, including the NMDA-receptor antagonist ketamine (Berman et al., 2000; DiazGranados et al., 2010; Zarate et al., 2006), and high-frequency electrical stimulation of the anterior cingulate cortex (ACC) and certain other brain areas (deep brain stimulation,

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DBS) (Delahoye and Holtzheimer, 2014; Hamani et al., 2011; McGrath et al., 2013; Mayberg, 2009).

The chronic mild stress (CMS) procedure is the most widely used animal model of depression (Antoniuk et al., 2019; Willner, 2017). In CMS experiments, rats or mice are subjected over several weeks to a constant bombardment of varying mild stressors, resulting in a wide range of behavioural and physiological changes characteristic of depression, which respond to chronic but not acute treatment with antidepressant drugs (Hill et al., 2012; Willner, 1997, 2017). Typically, a weekly sucrose intake or preference test, modelling the core symptom of anhedonia, is used to track the induction and remission of CMS effects (Antoniuk et al., 2019; Willner, 2017). Many recent studies have also employed the novel object recognition (NOR) test, a simple memory test that exploits the natural tendency of animals to explore novel objects (Ennaceur and Delacour, 1988). Memory in the NOR test is impaired by CMS and rescued by chronic treatment with antidepressant drugs (Elizalde et al., 2008; Liu et al., 2014; Llorente et al., 2011; Orsetti et al., 2007; Papp et al., 2016, 2017), and provides a simple model of the impairments of memory and executive functioning shown by depressed patients (Belzung et al., 2015; Carvalho et al., 2014; Malhi et al., 2015).

Like TRD patients, rats or mice subjected to CMS have similarly demonstrated a rapid reversal of CMS-induced anhedonia, impairment of NOR, and other depression-related behaviours in rats and mice following ketamine treatment (Maciel et al., 2018; Papp et al., 2017; Wen et al., 2019; Tornese et al., 2019) or DBS of the medial prefrontal cortex (mPFC) (Dourmes et al., 2013; Hamani et al., 2012; Lim et al., 2015; Veerakumar et al., 2014). In order to align these findings to the clinical situation, we developed a model of antidepressant treatment-resistance by implementing the CMS model in Wistar–Kyoto (WKY) rats. The WKY strain had long been considered to be resistant to antidepressant drug treatment, largely on the basis of acute studies using the forced swim test (Lahmame et al., 1997; Lopez-Rubalcava and Lucki, 2000; Tejani-Butt et al., 2003). We implemented the CMS model in WKY rats and demonstrated that they failed to recover from the effects of CMS following chronic treatment with different antidepressant drugs, but nevertheless did show recovery on a battery of behavioural tests (sucrose intake, NOR and the elevated plus maze (EPM)) following subchronic ketamine treatment or acute DBS of the mPFC. Acute DBS was also effective when administered to a subset of Wistar rats that failed to respond to antidepressant treatment (Papp et al., 2018; Willner et al., 2019).

In antidepressant-responsive strains there is evidence suggesting a role for the AMPA subtype of glutamate receptor in the mechanism of action of both antidepressant drugs (Ampuero et al., 2010; Barbon et al., 2011; Martinez-Turrillas et al., 2002; Neis et al., 2016; Park et al., 2018; Svenningsson et al., 2007) and novel treatments such as ketamine (Du et al., 2006; Maeng et al., 2008) and DBS (Jimenez-Sanchez et al., 2016a, 2016b). Here we have investigated the role of AMPA receptors in the mPFC in the action of chronic venlafaxine (VEN) in Wistars, and acute DBS in WKY. As in an earlier study of the role of dopamine receptors, using the same design (Papp et al., 2019a), we did not study DBS in Wistars because that would be uninformative in relation to TRD, and we did not administer VEN to WKY because they do not respond to antidepressant drugs. We asked whether antidepressant effects, in the CMS model, of VEN in Wistars and DBS,

would be blocked by the selective AMPA-receptor antagonist NBQX (Shimizu-Sasamata et al., 1996) or mimicked, in WKY, by the AMPA-receptor positive allosteric modulator CX-516 (Arai et al., 2002), when administered at the same site in the mPFC as DBS.

We also asked whether the antidepressant effect of DBS, in WKY rats, would be mimicked by optogenetic stimulation (OGS) at the same site in the mPFC. OGS has great potential as a technique to map out depression-relevant neural pathways (Biselli et al., 2019; Cheng et al., 2020). Previous studies have reported antidepressant-like effects of OGS in mice subjected to chronic social defeat (Bagot et al., 2015; Covington et al., 2010; Vialou et al., 2014), and in the forced swim or tail suspension test applied to normal mice (e.g. Hare et al., 2019; Son et al., 2018) or rats (Fuchikami et al., 2015; Jimenez-Sanchez et al., 2016a, 2016b). However, the effects of OGS have not previously been examined in a model of TRD. We report two experiments, asking whether an antidepressant-like effect of OGS in the NOR test would be blocked by NBQX (exactly paralleling the DBS experiment), and whether antidepressant-like effects would be elicited in WKY rats by OGS of the glutamatergic ventral hippocampal (vHPC)–mPFC pathway. This pathway has been described as ‘the weak link in psychiatric disorders’ (Godsil et al., 2013), and a failure to activate it could potentially explain antidepressant treatment resistance (Willner et al., 2014).

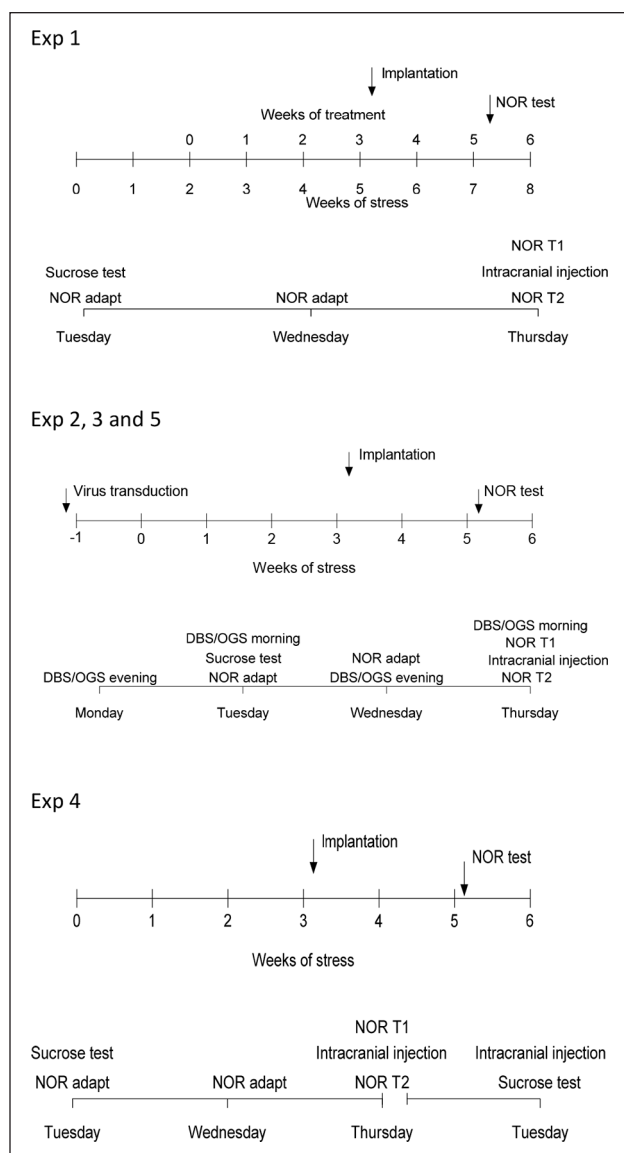
## Methods

### Subjects

Male Wistar and WKY rats (Charles River, Germany), weighing 100 g on arrival were housed singly with free access to food and water, and maintained on a 12-h light/dark cycle (lights on at 08.00 h) in conditions of constant temperature ( $22 \pm 20^\circ\text{C}$ ) and humidity ( $50 \pm 5\%$ ). At the time of the final baseline test before the onset of CMS (see below), mean body weights were 306 g in Wistars and 284 g in WKY. All procedures used conformed to the rules and principles of EEC Directive 86/609 and were approved by the Bioethical Committee at the Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland.

### Study design

Experiments 1–3 tested whether the AMPA antagonist NBQX would block the antidepressant effects of VEN in Wistars (experiment 1), or of DBS (experiment 2) or OGS (experiment 3) of mPFC in WKY. Experiments 4 and 5 tested whether the ampakine CX-516 (experiment 4) and OGS of the vHPC–mPFC pathway (experiment 5) would show antidepressant-like effects in WKY. In all experiments, stressed animals were subjected to CMS throughout the experiment, while controls (CON) were left undisturbed in their home cages, except for weekly sucrose intake tests, operative procedures and standard husbandry. During week 2 (experiment 5), 4 (experiments 2–4) or 6 (experiment 1) of stress, all animals received a unilateral implant in the left mPFC, which varied between experiments: an injection cannula in experiments 1 and 4, a cannula–electrode combination in experiment 2, a cannula–optical fibre combination in experiment 3, and an optical fibre in experiment 5. Two weeks later, during the final week of stress, animals were exposed to the NOR test.



**Figure 1.** Experimental timelines. For each experiment, the upper panel shows the timeline over weeks of the experiment, and the lower panel shows details of the final week. In the timeline for experiments 2, 3 and 5, virus transduction was applied in experiments 3 and 5 only. DBS, deep brain stimulation; NOR, novel object recognition (T1, time required to complete 15 s of exploration of both objects; T2, retention trial); OGS, optogenetic stimulation.

At the end of each experiment, animals were sacrificed and brains were processed to verify the location of implants and effectiveness of virus infection. Timelines for all five experiments are shown in Figure 1.

**Experiment 1.** Following 2 weeks of stress, Wistar rats in both CON and CMS groups ( $n = 48/\text{group}$ ) were treated daily for a further 5 weeks with either saline (SAL) or VEN (10 mg/kg) in a volume of 1 mL/kg body weight, intraperitoneal (IP) ( $n = 24/\text{group}$ ). They were implanted during week 6 of stress with a stainless-steel guide cannula. Immediately following the exposure trial in the

NOR test, three sub-groups ( $n = 8$ ) in each condition received intracranial injections of either saline or NBQX (1 or 4 nM).

**Experiment 2.** During week 4 of stress, WKY rats in both CON and CMS groups ( $n = 44/\text{group}$ ) were implanted with an electrode–cannula combination, through which they received either DBS or sham stimulation immediately preceding the final sucrose intake test and the NOR test ( $n = 20\text{--}22/\text{group}$ ). Within each of the four experimental conditions, three sub-groups ( $n = 6\text{--}8$ ) received intracranial injections of either saline or NBQX (1 or 4 nM) immediately following the exposure trial in the NOR test.

**Experiment 3.** During week 4 of stress, 5 weeks following virus transduction (see below), WKY rats in both CON and CMS groups ( $n = 48/\text{group}$ ) were implanted with an optical fibre–cannula combination, through which they received either OGS or sham stimulation immediately preceding the final sucrose intake test and the NOR test ( $n = 24/\text{group}$ ). Within each of the four experimental conditions, three sub-groups ( $n = 8$ ) received intracranial injections of either saline or NBQX (1 or 4 nM) immediately following the exposure trial in the NOR test.

**Experiment 4.** During week 4 of stress, WKY rats in both CON and CMS groups ( $n = 24/\text{group}$ ) were implanted with a stainless-steel guide cannula. Within each condition, three sub-groups ( $n = 8$ ) received intracranial injections of either saline or CX-516 (0.05 or 0.15  $\mu\text{g}$ ) immediately following the exposure trial in the NOR test. A final sucrose test was conducted four days later, preceded (15 min before the start) by a second injection of the same dose of CX-516.

**Experiment 5.** During week 4 of stress, 5 weeks following virus transduction (see below), WKY rats in both CON and CMS groups ( $n = 16/\text{group}$ ) were implanted with an optic fibre through which they received either OGS or sham stimulation immediately preceding the final sucrose intake test and the NOR test ( $n = 8/\text{group}$ ). For comparability with a different set of experiments (to be reported elsewhere), these animals were also tested on the EPM, using a standard methodology (Papp et al., 2018).

### Behavioural procedures

**CMS.** The CMS procedure was conducted as previously described (Papp, 2012). Briefly, after 3 weeks of habituation to laboratory and housing conditions, the animals were trained to consume a 1% sucrose solution in six baseline tests conducted once weekly in the home cage. After 14 h food and water deprivation, the animals were presented with a freshly prepared 1% sucrose solution for 1 h. Sucrose intake was calculated by weighing bottles before and after the test. Subsequently, sucrose consumption was monitored once weekly, under similar conditions, until the end of the study.

Each week of the stress regime consisted of: two periods of food or water deprivation, two periods of 45° cage tilt, two periods of intermittent illumination (light on and off every 2 h), two periods of soiled cage (250 mL water in sawdust bedding), one period of paired housing, two periods of low intensity stroboscopic illumination (150 flashes/min) and three periods of no stress. The duration of all stressors was 10–14 h and they were

applied individually and continuously, day and night. CON animals were housed in separate rooms and were deprived of food and water for 14 h before each sucrose test, but otherwise food and water were freely available.

**NOR test.** The animals were tested in an opaque circular open field (100 cm in diameter, 35 cm high, floor divided into painted 16-cm squares). After a period of 2 days adaptation to the open field (10 min daily), the animals were allowed to explore two identical cylinder-shaped white objects (7 cm in diameter, 11 cm high) for the time required to complete 15 s of exploration of both objects (T1 session). In the retention trial (T2 session) conducted one hour later, one of the objects presented previously was replaced by a novel prism-shaped black object (5 cm wide, 14 cm high). Rats were returned to the open field for 5 min and the duration of exploration of each object (i.e. sitting in close proximity to the objects, sniffing or touching them) was recorded by a trained observer who was blind to treatments. A recognition index was calculated according to the formula: time of novel object exploration minus time of familiar object exploration, divided by total exploration time (novel plus familiar objects) (Akkerman et al., 2012). During NOR sessions, the number of line crossings was recorded as a measure of locomotor activity.

### Stimulation and injection procedures

**Surgery.** Animals were anaesthetized with pentobarbital (60 mg/kg IP) and placed in a stereotaxic apparatus (Stoelting Co., Wood Dale, IL, USA), for implantation in the PFC with either a custom-made unilateral stainless-steel guide cannula (experiments 1 and 4), a combined electrode–cannula implant (experiment 2), a combined optical fibre–cannula implant (experiment 3), or an optical fibre (experiment 5). The implant was connected to a plastic pedestal and was fixed to the skull with dental cement (Adhesor Carboline, SpofaDental, Jicin, Czech Republic).

The guide cannulae (experiments 1–4), which had an external diameter of 0.6 mm and were fitted with stainless steel obturators to prevent occlusion, were aimed at the left ventromedial (vm)-PFC (anteroposterior (AP) +3.0 mm, lateral (L) +0.7 mm, dorsoventral DV –2.8 mm from Bregma) according to the atlas of Paxinos and Watson (1998). In experiment 2, a monopolar stainless-steel stimulating electrode (model C315G-MS303/2, Plastics One Inc., Roanoke, VA, USA) consisted of two wires; one wire (250 µm in diameter, 0.75 mm of exposed surface), which was attached to the guide cannula and extended 1.4 mm below it, served as the cathode; the second wire had an epidural screw attached and was used as the anode. In experiments 3 and 5 an optical fibre was implanted (model DFC\_200/230-0.48\_5mm\_ZF2.5(G)\_FLT, Doric Lenses Inc., Quebec, Canada), which in experiment 3 was attached to the guide cannula with its end extending 0.75 mm below it, and in experiment 5 extended to the same depth (AP +3.0 mm, L +0.7 mm, DV –3.5 mm).

**DBS.** After a 2-week post-operative recovery period, animals received stimulation at the following parameters: amplitude 250 µA, frequency 130 Hz; pulse width 90 µs. Stimulation was administered using a custom-made multi-channel stimulator and digital-to-analogue converters, connected to the animals through bipolar extension cables and commutators (Plastics One Inc., Roanoke, VA, USA). Two 2-h DBS sessions were conducted, one

on the previous evening and one on the morning before each of the week 5 sucrose intake test and the NOR test T1 session. The stimulation parameters and the schedule of DBS administration were as used in previous studies (Papp et al., 2018; Willner et al., 2019). Animals in the sham group were treated in the same way as DBS animals but the stimulator was switched off.

**OGS.** During the week before the penultimate baseline sucrose test animals received intra-PFC (experiment 3) or intra-HPC (experiment 5) administration of adeno-associated viruses (AAVs) inducing channelrhodopsin-2 (ChR2) and EYFP gene expression non-selectively in all neurons (AAV5-hSyn-ChR2-EYFP). AAVs were obtained from the University of North Carolina Viral Core. Rats with virally delivered EYFP only (AAV5-hSyn-EYFP) were used as sham controls.

For viral infusion, animals were anaesthetized with pentobarbital (60 mg/kg, IP) and placed in a stereotaxic apparatus (Stoelting Co., Wood Dale, IL, USA). A small skin incision at the top of the scalp and a small hole in the skull were made above the left mPFC (experiment 3) or vHPC (experiment 5), followed by AAV infusion (0.1 µL/min; total volume 0.5 µL;  $4.4 \times 10^{12}$  virus molecules/mL) into the PFC (AP +3.0, L +0.7 DV –3.5 mm from Bregma) or HPC (AP –5.3, L +5.5, DV –7.5), according to the atlas of Paxinos and Watson (1998). Infusions were made using an infusion pump and 2 µL Hamilton syringes. 5 min later, the skin was sutured and the animals were transferred to their home cages.

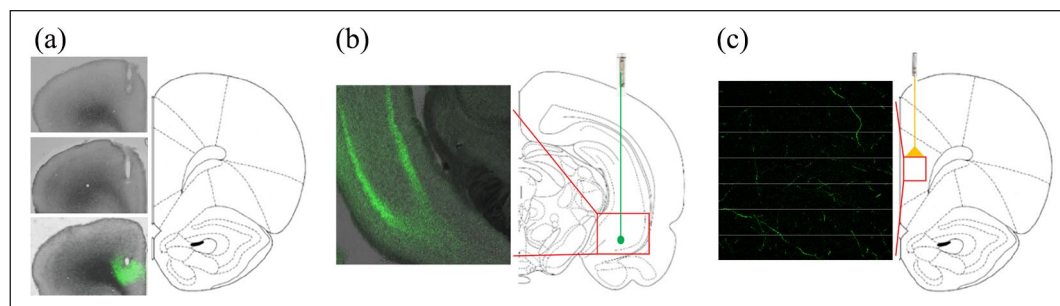
Five weeks after virus transduction, animals were implanted with an optical fibre (see above) and 2 weeks later they received a total of four 60-min sessions of stimulation with blue light pulses (473 nm, 15 ms light pulses at 20 Hz; 1 min on and 1 min off for 30 cycles). For each session, the rats were connected to a laser source (473 nm, with power density of approximately 5 mW/mm<sup>2</sup> at the fiber tip) via fibre-optic patchcord (Ø200 µm Core, 0.37 NA, Doric Lenses Inc., Quebec, Canada), fibre-optic rotary joint (Doric Lenses Inc., Quebec, Canada) and fibre-optic patchcord (Ø200 µm Core, 0.22 NA, CNI Optoelectronics Tech. Co. Ltd, Changchun, China). Two 1-h OGS sessions were conducted before each of final sucrose intake test and the NOR test T1 session (Figure 1), one on the previous evening and one in the morning ending 15 min before the test. The stimulation parameters and the schedule of OGS administration were based on the study of Fuchikami et al. (2015) and our preliminary studies

**Intracranial injections.** For intracranial injections, a stainless-steel internal cannula (0.4 mm external diameter, extending 0.7 mm below the guide cannula) was inserted into the guide cannula and 0.5 µL of solution was infused. Infusions of NBQX (experiments 1–3) or CX-516 (experiment 4) were made over 1 min, using an infusion pump and 2 µL Hamilton syringes. The internal cannulae were left in place for 1 min to avoid backflow of the infusion.

### Verification of implants and virus infection

At the end of each experiment animals were sacrificed by decapitation and the correct placement of infusion and stimulation cannulae was verified in frozen coronal sections of brains cut throughout the target areas to visualize the cannula routes and the injection sites. Animals in which the cannula/electrode tips were found to be outside the target areas, which amounted to 2.5% overall, were





**Figure 2.** (a) The three panels to the left show representative tracks of the guide cannulae for intracranial infusions (top), deep brain stimulation electrode (middle) and optogenetic stimulation (OGS) (bottom). In each case, the injection site was 0.70 mm ventral to the guide cannula tip. The green area in the bottom panel shows AAV5-hSyn-ChR2-EYFP virus transduction (green) in the prefrontal cortex (PFC). (b) AAV5-hSyn-ChR2-EYFP virus transduction (green) in the ventral hippocampus (vHPC). The probe in the right panel indicates the site of injection. (c) AAV5-hSyn-ChR2-EYFP virus infection (green) in terminals of the vHPC-medial PFC pathway following virus transduction in the vHPC. The right panel shows the location within prefrontal cortex, with the probe indicating the site of OGS.

excluded from the data analysis. The correct placement of the optical fibre and effectiveness of the virus transduction were verified in coronal brain sections (40  $\mu$ m) with the use of confocal microscopy Leica TCS SP8 WLL (Leica Microsystems, magnification 10 $\times$ ). Representative placement data are shown in Figure 2.

### Drugs

VEN (Carbosynth Ltd, Compton, Berkshire, UK), NBQX (Tocris Bioscience, Avonmouth, Bristol, UK) and CX-516 (Adooq Bioscience LLC, Irvine, CA, USA) were dissolved in 0.9% sterile SAL, which was used for VEH administration. The doses were either as used previously (Papp et al., 2018, 2016, 2017, 2019a) or based on our preliminary studies.

### Statistical analysis

NOR data from experiments 1–3 were analysed in a single 4-way analysis of variance (anova), with the factors Stress (CON v. CMS), antidepressant (CON or active treatment), dose of NBQX, and experiment. A 3-way anova, omitting NBQX, was used for the sucrose intake data. Data from experiment 4 were analysed by a 2-way anova with the factors stress and dose of CX-516, and a 1-way anova of dose effects. Data from experiment 5 were analysed by a 2-way anova with the factors stress and OGS (real or sham).

## Results

### AMPA receptors mediate pro-cognitive effects of antidepressant treatments (experiments 1–3)

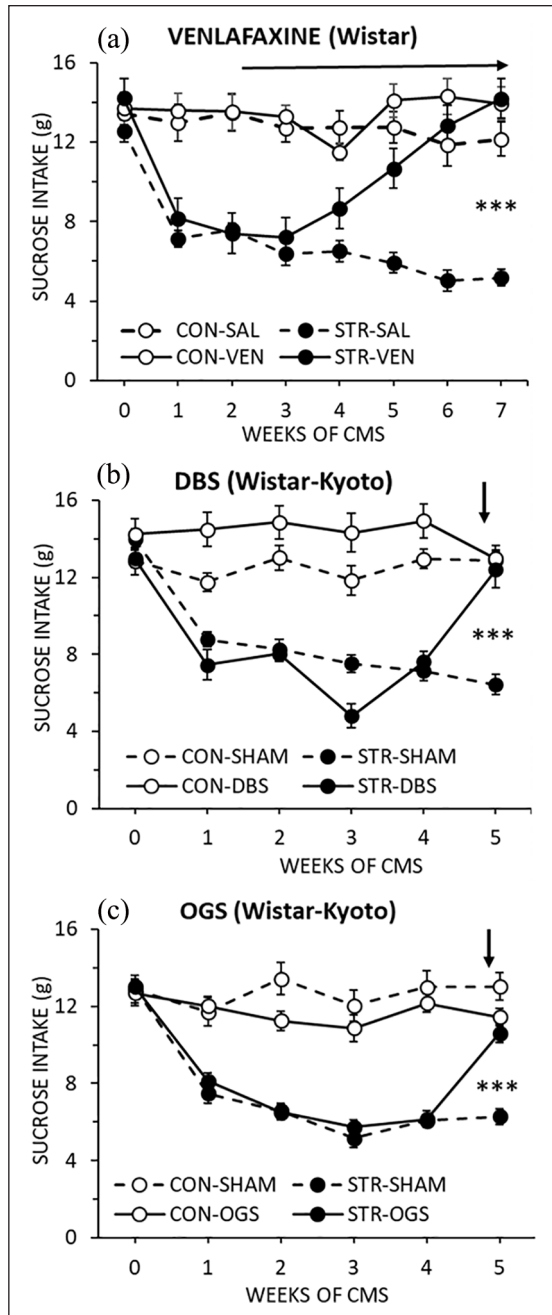
Sucrose intake was decreased by CMS and restored gradually by chronic treatment with VEN in Wistar rats (Figure 3(a)) or acutely by DBS (Figure 3(b)) or OGS (Figure 3(c)) of the mPFC in WKY rats. Anova of performance on the final test day confirmed a significant CMS  $\times$  treatment interaction ( $F(1, 244) = 70.3, p < 0.001$ ) that did not differ between experiments (3-way interaction:  $F(2, 244) = 0.31, NS$ ).

Performance in the NOR test was essentially the same across the three experiments. NOR was abolished by CMS and restored by VEN in Wistars (Figure 4(a)) or following DBS (Figure 4(b)) or OGS (Figure 4(c)) in WKY. The higher dose of NBQX (4 nM) abolished NOR in all groups. However, the lower dose of NBQX (1 nM) was without effect in control groups, but in all three experiments selectively impaired NOR following recovery from stress. Anova confirmed a significant stress  $\times$  antidepressant  $\times$  NBQX interaction ( $F(2, 252) = 6.61, p < 0.002$ ), and that there were no significant differences between experiments in any effects or interactions involving CMS, antidepressant treatment or NBQX (max  $F = 0.64, NS$ ).

Both exploratory activity (Figure 5a) and locomotor activity (Figure 5(b)) in the NOR test were higher in Wistars (VEN experiment) than in WKY (DBS and OGS experiments) ( $F(2, 244) = 110.1$  and  $234.3$ , respectively,  $p < 0.001$ ), and both measures were increased by DBS, but not by OGS or VEN (experiment  $\times$  treatment interaction:  $F(2, 244) = 3.88, p < 0.02$  and  $11.5, p < 0.001$ , respectively). Otherwise, there were minimal effects on exploratory and locomotor activities. Exploratory activity was marginally decreased by CMS ( $F(1, 244) = 3.25, p = 0.073$ ), but the main effect of NBQX and all interactions involving NBQX were nonsignificant (max  $F = 1.41, NS$ ). For locomotor activity, there was a marginally significant NBQX  $\times$  experiment interaction ( $F(4, 244) = 2.54, p = 0.04$ ), but the main effect of NBQX was not significant in any of the individual experiments; all other interactions involving NBQX, and all effects involving CMS, were nonsignificant (main effect of CMS:  $F(1, 244) = 1.63$ ; else, max  $F = 1.33$ ). Notably, the higher dose of NBQX, which in all three experiments abolished NOR in both controls and antidepressant-treated animals, had no significant effect on exploratory or locomotor behaviour in any group.

### Antidepressant-like effects of the ampakine CX-516 (experiment 4)

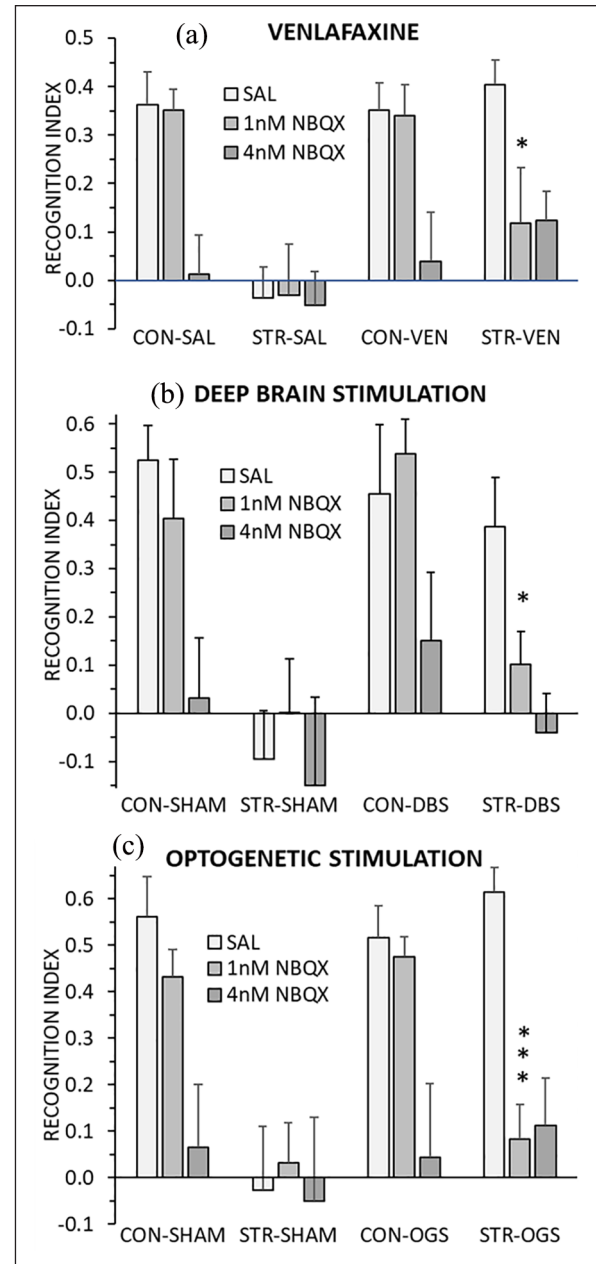
CX-516 caused a dose-dependent restoration of NOR in stressed animals, with no effect in the non-stressed group (Figure 6(a)), as reflected in a significant dose  $\times$  stress interaction ( $F(1, 42) = 4.03, p < 0.025$ ). The higher dose of CX-516 increased exploratory behaviour in the NOR test (Figure 6(b)) in both controls and stressed



**Figure 3.** Sucrose intake was suppressed by chronic mild stress (CMS) and restored by (a) chronic venlafaxine (VEN) in Wistars (horizontal arrow) or (b) by acute deep brain stimulation (DBS) or (c) optogenetic stimulation (OGS) in Wistar-Kyoto (WKY) (vertical arrow). Values are means  $\pm$  SEM;  $n = 6-8$ /group (DBS) or 8/group (VEN and OGS). STR, stressed; CON, control.

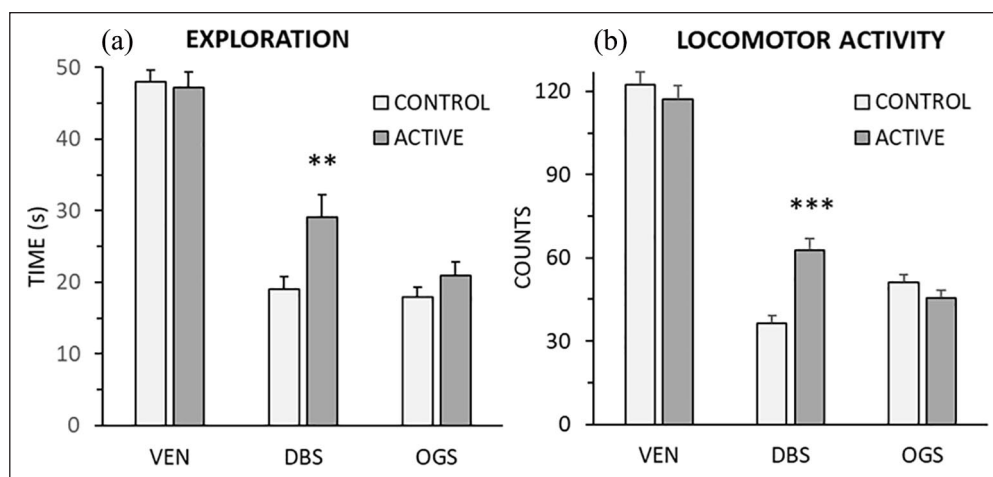
animals ( $F(2, 42) = 4.24, p < 0.025$ ). There were no other significant main effects or interactions for either exploratory behaviour (Figure 6(b)) or locomotor activity (Figure 6(c)) (max  $F = 0.82$ ).

Acute administration of CX-516 into the mPFC of WKY rats prior to the final sucrose intake test increased sucrose intake in stressed animals at both doses (Figure 5(d)). At the higher dose, sucrose intake was also slightly higher in non-stressed controls.



**Figure 4.** Novel object recognition (NOR) was abolished by chronic mild stress (CMS) and restored by (a) chronic administration of venlafaxine (VEN) in Wistars or by (b) acute deep brain stimulation (DBS) or (c) optogenetic stimulation (OGS) of medial prefrontal cortex in WKY. The higher dose of NBQX abolished NOR in all groups, but in all three experiments, the lower dose of NBQX selectively impaired NOR in antidepressant-treated stressed animals:  $*p < 0.05$ ,  $***p < 0.001$  relative to the corresponding saline (SAL)-treated group. Values are means  $\pm$  SEM;  $n = 6-8$ /group (DBS) or 8/group (VEN and OGS). STR, stressed; CON, control.

As a result, the dose  $\times$  stress interaction was not significant ( $F(2, 42) = 1.41$ , NS); however, separate exploratory analyses confirmed that the effect of dose was significant in stressed animals ( $F(2, 21) = 8.23, p < 0.002$ ) but not in controls ( $F(2, 21) = 0.35$ ).



**Figure 5.** Effects of chronic venlafaxine (VEN) in Wistars, or acute deep brain stimulation (DBS) or optogenetic stimulation (OGS) of medial prefrontal cortex in WKY, on (a) exploratory and (b) locomotor behaviour in the novel object recognition test. DBS selectively increased both measures, relative to sham stimulation. \*\* $p < 0.01$ , \*\*\* $p < 0.005$ . White bars: control treatments (saline or sham stimulation); grey bars: active treatments (VEN, DBS or OGS). Values are means (averaged across CMS and NBQX sub-groups)  $\pm$  SEM;  $n = 42\text{--}48/\text{group}$ .

### Acute OGS of the vHPC–mPFC pathway does not reverse effects of CMS (experiment 5)

OGS of vHPC terminals in the PFC did not reverse the effect of CMS to decrease sucrose intake (Figure 7(a)), impair NOR (Figure 7(b)), or elicit an anxiogenic response in the EPM (results not shown) (max  $F(1,28) = 0.44$  for main effects of OGS and interactions with CMS). However, in both controls and stressed animals, OGS of vHPC terminals increased exploratory behaviour (Figure 7(c)) ( $F(1, 28) = 8.38, p < 0.005$ ) and locomotor activity (Figure 7(d)) ( $F(1, 28) = 8.38, 7.65, p < 0.01$ ) in the NOR test.

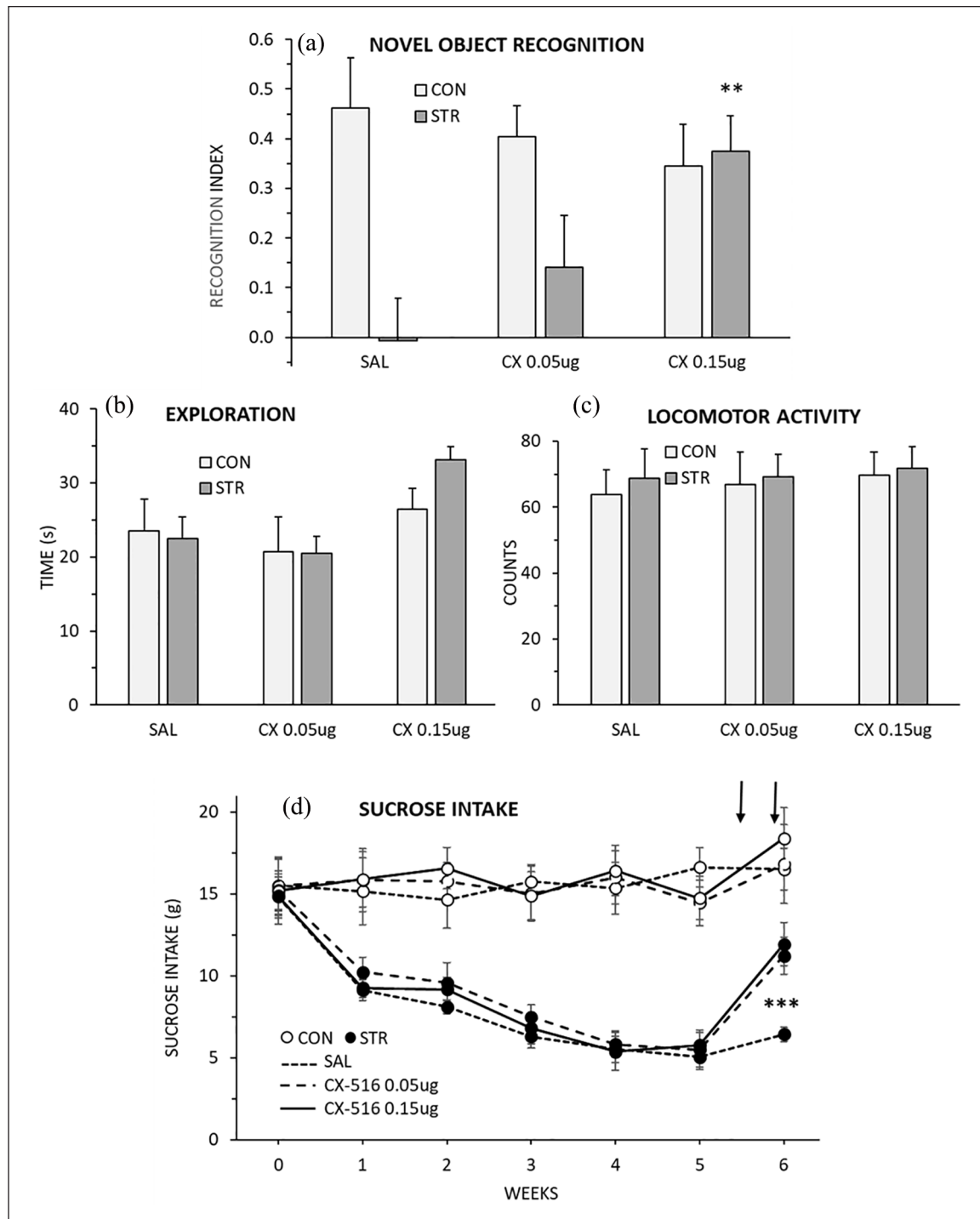
## Discussion

As in previous studies, sucrose intake and NOR were suppressed by CMS, and restored by the antidepressant VEN in Wistars (Papp et al., 2017, 2019a), and by DBS of the mPFC in antidepressant-resistant WKY (Papp et al., 2018, Willner et al., 2019). We now report that OGS and the AMPA receptor positive allosteric modulator CX-516, administered at the same site in the mPFC, also have antidepressant-like effects on the same two measures in WKY rats subjected to CMS. Conversely, a low dose of the AMPA receptor antagonist NBQX, also at the same site, selectively blocked the procognitive effect, in the NOR test, of VEN, DBS and OGS. Taken together with the antidepressant-like effects of CX-516, these effects of NBQX strongly suggest that activation of AMPA receptors in the mPFC represents a common pathway for the antidepressant effects of both conventional (VEN) and novel (DBS, OGS) antidepressant modalities, in both antidepressant responsive (Wistar) and antidepressant-resistant (WKY) rats.

In addition to their antidepressant-like effects, DBS and CX-516 also had minor non-specific effects on exploration (DBS and CX-516) and locomotor activity (DBS only) in the NOR test, but OGS did not share these effects. A likely explanation for these discrepancies is that DBS excites both intrinsic cells and afferents to PFC in the infected region, whereas OGS of mPFC excites only intrinsic cells. This latter explanation is suggested by

the fact that OGS of vHPC afferents to HPC had the same non-specific effects as DBS, to increase exploratory behaviour and locomotor activity. That is, as summarised in Figure 8, DBS showed two sets of effects, equivalent to those of both OGS of mPFC intrinsic cells (restoration of sucrose intake and NOR in stressed animals), and OGS of the vHPC–mPFC pathway (increased exploration and locomotion in both stressed animals and controls).

The higher dose of NBQX (4 nM) abolished NOR in all nine groups tested. An early study reported that NOR was unaffected by systemic administration of NBQX, which impaired locomotor activity at higher doses (Pitsikas et al., 2002). However, more recent studies employing intracranial administration have reported impairment of NOR by NBQX injected into perirhinal cortex (Malkova et al., 2015; Winters and Bussey, 2005) or hippocampus (Iwamura et al., 2016; Schiapparelli et al., 2006). We are unaware of any previous report of impairment of NOR by an AMPA-receptor agonist administered within the mPFC. Indeed, there is some scepticism in the literature about an involvement of mPFC in NOR, as distinct from certain other recognition memory tasks (e.g. de Souza Silva et al., 2016; Morici et al., 2015; Warburton and Brown, 2010), notwithstanding that there is a robust literature describing modulation of NOR by interventions within the mPFC (De Bundel et al., 2013; Pezze et al., 2015; Rossato et al. 2013; Watson et al., 2012), including our own earlier studies (Papp et al., 2017, 2018, 2019a; Willner et al., 2019). The effect of NBQX to suppress NOR was behaviourally specific, because overall object exploration and locomotor activity in the NOR test were unaffected. An effect on memory encoding can be excluded because NBQX was administered after the exposure trial in the NOR, suggesting a specific role for AMPA receptors in the mPFC in consolidation or retrieval of memory for a single object. It can also be inferred that performance in the NOR test is dependent on information processing within the mPFC, rather than as a result of activity in the downstream pathways that are also activated by DBS and OGS.

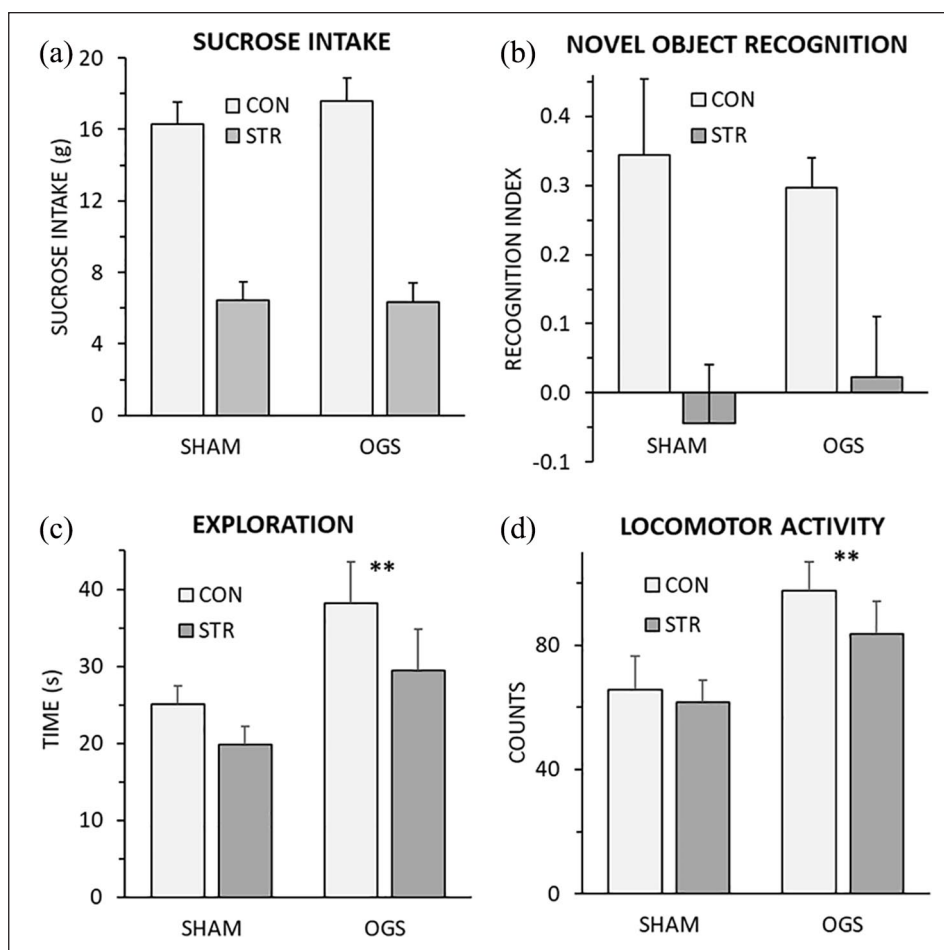


**Figure 6.** Effects of chronic mild stress (CMS) and CX-516 on (a) novel object recognition (NOR), (b) exploratory and (c) locomotor activity in the NOR test, and (d) sucrose intake. CX-516 dose-dependently restored NOR (a) and partially restored sucrose intake at both doses (d) in stressed (STR) animals, without significant effects in controls (CON). Vertical arrows (d) show the timing of CX-516 injections into the prefrontal cortex immediately prior to the sucrose intake test and, 4 days earlier, following the exposure trial in the NOR test. Values are means  $\pm$  SEM;  $n = 8$ ; \*\* $p < 0.01$ , \*\*\* $p < 0.001$  relatively to saline (SAL)-treated animals.

In contrast to the general suppression of NOR by the higher dose of NBQX, the lower dose (1 nM) selectively abolished NOR in stressed animals treated with VEN, DBS or OGS, with no effect in non-stressed controls, both untreated and treated.

This implies that both chronic VEN (in Wistars) and acute DBS or OGS (in WKY) sensitize AMPA-mediated transmission within the mPFC. This inference is consistent with results of earlier studies using less ecologically valid experimental procedures



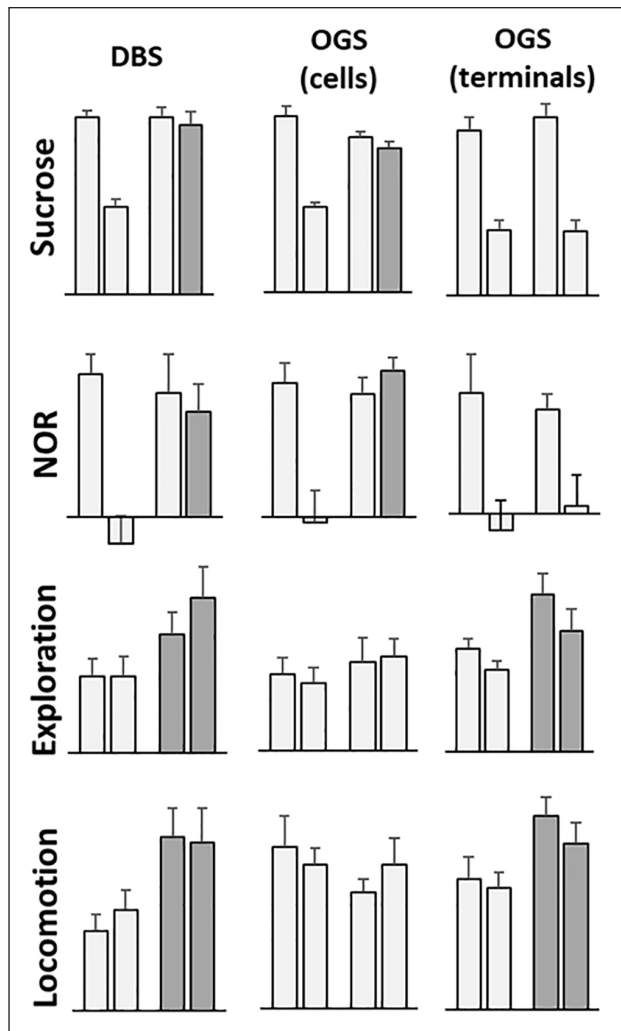


**Figure 7.** Optogenetic stimulation (OGS) of the ventral hippocampus–medial prefrontal cortex pathway did not reverse effects of chronic mild stress on (a) sucrose intake or (b) novel object recognition. OGS non-specifically increased (c) exploration and (d) locomotor activity in the NOR test, in both control (CON) and stressed (STR) animals; Values are means + SEM;  $n = 8$ ;  $**p < 0.01$ , relative to sham-stimulated groups.

applied to antidepressant-responsive strains. For example, in normal or olfactory bulbectomized rats, DBS increased the efflux of glutamate and synthesis of the GluA1 AMPA receptor subunit in the vm-PFC, local infusion of NBQX blocked the effects of DBS, and local infusions of AMPA also had an antidepressant-like effect (Jiménez-Sánchez et al., 2016a, 2016b). Like the present data, these results suggest that the antidepressant effect of acute vm-PFC DBS in rats is dependent on increased glutamate outflow in the mPFC, acting locally through AMPA receptors. A similar mechanism has been proposed for the rapid antidepressant action of ketamine (Du et al., 2006; Maeng et al., 2008), although alternative mechanisms are also under consideration (Ago et al., 2019; Aleksandrova et al., 2017; Kadriu et al., 2019; Yang et al., 2019). The mechanism of these effects may involve the GluA1 subunit of the AMPA receptor (Freudenberg et al., 2015), expression of which is known to be decreased in PFC (and other brain regions) by CMS (Toth et al., 2008) or chronic restraint stress (Yuen et al., 2012), and increased not only by DBS (Jiménez-Sánchez et al., 2016a), but also by chronic treatment with antidepressant drugs (Ampuero et al., 2010; Barbon et al., 2011; Martínez-Turrillas et al., 2002; Neis et al., 2016). Other studies have reported that phosphorylation of the AMPA

receptor, which is known to regulate synaptic efficacy (Huganir et al., 2013), was decreased by chronic social defeat (Park et al., 2018) and restored by fluoxetine (Park et al., 2018; Svenningsson et al., 2007). However, it is important to note that although these studies support the concept that AMPA-receptor activation in the mPFC may underlie the actions of diverse modalities of antidepressant treatment, they demonstrate a restoration of AMPA function to normal levels in stressed animals, and do not as yet explain either how antidepressant drugs or brain stimulation generate this effect, or why antidepressant-treated stressed animals are more sensitive to NBQX than non-stressed controls, while behaving similarly in the absence of NBQX.

A limitation of the study is that we only examined the effect of NBQX in the NOR test, but the effect of CX-516 to reverse the impairment of sucrose drinking by CMS suggests that the mechanism proposed may have a wider relevance. However, CX-516 has previously been shown not only to reverse phencyclidine-induced impairment of NOR when administered systemically (Damgaard et al., 2010), but also to improve memory in an object-in-place task in normal animals when injected at essentially the same site in mPFC as used here (Benn et al., 2016). Consequently, we cannot exclude that the lack of effect of



**Figure 8.** Comparison of the behavioural effects of deep brain (DBS) and optogenetic stimulation (OGS) of medial prefrontal cortex (mPFC). The four rows of data, reading down, represent the different behaviours measured: sucrose intake; novel object recognition (NOR); exploratory behaviour; locomotor activity. The three columns, reading across, summarize effects of DBS (left column), OGS of intrinsic cells in mPFC (middle column), and OGS of hippocampal (HPC) afferent terminals (right column). Within each panel, the four columns, reading left to right, represent sham-stimulated controls, sham-stimulated chronic mild stress (CMS), DBS or OGS, and DBS/OGS + CMS (redrawn from data shown in Figures 3–5 and 7). The dark bars show significant effects of DBS or OGS. For sucrose intake (top row) and NOR (second row), both DBS and OGS of cells specifically reversed effects of CMS, while exploratory behaviour (third row) and locomotor activity (bottom row), were non-specifically increased by both DBS and OGS of terminals (OGS). In summary, DBS has two sets of effects, that are mimicked by OGS of either cells or terminals.

CX-516 in the NOR test in non-stressed animals could result from a ceiling effect rather than providing evidence for a specific effect in stressed animals.

There is some uncertainty about the precise location within the mPFC at which stimulation elicits antidepressant-like effects.

An initial study, in mice subjected to chronic social defeat (Covington et al., 2010), and subsequent studies from the same group (Bagot et al., 2015; Vialou et al., 2014), reported that OGS elicited a recovery of social interaction and sucrose preference from placements within the prelimbic cortex (PLC). However, other studies in mice (e.g. Hare et al., 2019; Son et al., 2018) or rats (Fuchikami et al., 2015; Jiménez-Sánchez et al., 2016a, 2016b), using acute procedures such as the forced swim or tail suspension test in non-stressed animals, elicited antidepressant-like effects from placements within the infralimbic cortex (ILC). Indeed, Fuchikami et al. (2015) reported that OGS was ineffective, in Sprague–Dawley rats, when the probes were located within the PLC. As in our earlier DBS studies (Papp et al., 2018, 2019a; Willner et al., 2019), the probes used here for both DBS and OGS were located within the PLC (Figure 2(a)), at the coordinates where DBS was first reported to elicit antidepressant-like effects (Hamani et al., 2010a, 2010b). The area of virus infection in the study of Fuchikami et al. (2015) was located more dorsally within the PLC, while their ILC virus infusions also infected the ventral portion of the PLC (see Fuchikami et al., 2015, figs S5, S6). Whether there are one or two hot spots for DBS/OGS of mPFC remains to be resolved: potentially relevant factors include the level of stress, the particular behavioural tests used, stimulation parameters, or whether stimulation was delivered before or during the test.

Optogenetic and chemogenetic techniques have been used to map out a network of pathways potentially involved in antidepressant action, including efferent pathways from mPFC to the nucleus accumbens, dorsal raphe nucleus and lateral habenula, and afferent pathways to the mPFC from the ventral tegmental area and vHPC (Biselli et al., 2019; Cheng et al., 2020; Muir et al., 2019). The common action of NBQX to block the antidepressant actions of VEN in Wistars and DBS or OGS in WKY has the important implication that antidepressant resistance in WKY is likely to result from problems on the afferent side that prevent antidepressant drugs from activating the mPFC. The vHPC–mPFC pathway is a strong candidate to mediate such an effect. Stress-induced inactivation of the vHPC, with a consequent loss of vHPC–mPFC transmission, has been proposed as the basis of depressive psychopathology (e.g. Willner and Belzung, 2015), and one study has reported that activation of this pathway was both necessary and sufficient for the antidepressant-like effect of ketamine in the mouse forced swim test (Carreno et al., 2016). The restoration of mPFC afferent activity by antidepressant drugs may be compromised in WKY by differences in hippocampal dynamics: a genomic screening study found that the ratio of ventral to dorsal hippocampal expression of depression-related genes was lower in WKY, relative to drug-responsive Wistars, for 11 of the 22 genes examined (Papp et al., 2019b). We therefore hypothesized that antidepressant resistance in WKY rats might be caused by insufficiency of vHPC–mPFC transmission.

An initial test of this hypothesis was negative: unlike OGS of cell bodies in mPFC, OGS of vHPC afferents to mPFC did not elicit antidepressant-like effects, notwithstanding that the non-specific activating effects of stimulating vHPC terminals confirm the effectiveness of the stimulation. However, this does not exclude that activity in the vHPC–mPFC pathway may be necessary for antidepressant drugs to exert their neuroplastic effects during chronic treatment, and this hypothesis is currently under investigation. Additionally, the negative effect of vHPC–mPFC

stimulation is informative as regards the anatomical basis of the antidepressant effect of DBS. It is known that the vHPC–mPFC pathway is glutamatergic and that activation of the pathway stimulates AMPA receptors in PFC (Jay et al., 1992; Parent et al., 2010). Therefore, it can be inferred from the lack of antidepressant-like effect of vHPC–mPFC stimulation that the antidepressant-like effects of DBS and CX-516 are mediated by a discrete set of AMPA synapses that are anatomically separate from those innervated by HPC afferents. The latter are located in both superficial and deep layers of infralimbic cortex, but are more localized to deep layers of prelimbic cortex (Liu and Carter, 2018).

We have previously reported that a dopamine D2 receptor antagonist, administered at the same site in the mPFC, blocked the recovery of NOR in stressed Wistar rats treated chronically with VEN, but had no impact on the recovery of NOR in stressed WKY rats treated with DBS, under the identical conditions to those used here (Papp et al., 2019a). A single set of studies has reported that the effect of DBS in naïve rats subjected to the forced swim test was reversed by NBQX and by blockade of the mammalian target of rapamycin (mTOR), an intracellular mediator downstream from AMPA receptors (Jiménez-Sánchez et al., 2016a, 2016b). We are not aware of any other pharmacological studies of the mechanism of action of DBS. Similarly, while there is an extensive literature on depression-relevant anatomical pathways activated by OGS of the mPFC, as well as related chemogenetic studies (Biselli et al., 2019; Cheng et al., 2020; Muir et al., 2019), we are unaware of any previous pharmacological study of the mechanisms underlying the effects of OGS of the mPFC. We have used a well-validated model of depression to demonstrate that activation of a population of AMPA receptors is necessary and sufficient for antidepressant actions of both DBS and OGS of the mPFC in a validated antidepressant drug-resistant rat strain. The ecological relevance of the behavioural paradigm used supports the growing tide of opinion promoting glutamatergic strategies as a potential solution to the problem of TRD.

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